

CLAIMS

We claim:

5 *Sub A4*

1. A method, comprising:

10 a) providing:

15 i) a first sample comprising a plurality of proteins;

15 ii) a second sample comprising a plurality of proteins;

15 iii) a separating apparatus, wherein said separating apparatus separates proteins based on a physical property;

15 iv) a mass spectroscopy apparatus; and

15 b) treating said first and second samples with said separating apparatus to produce a first separated protein sample and a second separated protein sample, wherein said first and second separated protein samples are collected from said separating apparatus in a plurality of fractions, each of said fractions defined by a physical property; and

15 c) analyzing said plurality of fractions from each of said first and second separated protein samples with said mass spectroscopy apparatus to produce a protein profile map for each of said first and second samples, wherein said protein profile maps display protein abundance and mass of said first protein sample and said second protein sample.

20 *Sub B1*

20 2. The method of Claim 1, further comprising an automated sample handling device operably linked to said separating apparatus and said mass spectroscopy apparatus, wherein said sample handling device transfers said first and second samples to said separating apparatus, and wherein said sample handling device transfers said first and second separated protein samples from said separating apparatus to said mass spectroscopy apparatus.

25 3. The method of Claim 2, further comprising a centralized control network operably linked to said automated sample handling device, said separating

apparatus, and said mass spectroscopy apparatus, wherein said centralized control network controls the operations of said automated sample handling device, said separating apparatus, and said mass spectroscopy apparatus.

4. The method of Claim 3, wherein said centralized control network
5 comprises computer memory and a computer processor.

5. The method of Claim 1, wherein said first sample comprises a cell lysate from a first cell type and said second sample comprises a cell lysate from second cell type.

6. The method of Claim 5, wherein said first cell type is a cancerous cell
10 type and said second cell type is a non-cancerous cell type.

7. The method of Claim 1, wherein said protein abundance is expressed as bands of varying intensity.

Sub P2 8. The method of Claim 7, wherein said protein abundance is expressed as bands of different colors.

15 9. The method of Claim 1, wherein said protein abundance and mass are indicative of the cell type of said protein sample.

Sub B1 10. The method of Claim 1, further comprising the step of d) determining the identity of individual bands on said protein profile map.

20 11. The method of Claim 6, further comprising the step of treating said first sample with an external agent prior to treating said first and second samples with said separating apparatus.

12. The method of Claim 11, wherein said external agent comprises estradiol.

13. The method of Claim 2, wherein said automated sample handling device comprises a switchable, multi-channel valve.

5 14. The method of Claim 1, wherein said first and second samples further comprise a buffer, wherein said plurality of proteins are solubilized in said buffer and wherein said buffer is compatible with said separating apparatus and said mass spectroscopy apparatus.

10 *Sub A3* 15. The method of Claim 16, wherein said buffer comprises a compound of the formula n-octyl C_6C_{12} glycopyranoside.

15 *Sub B1* 16. The method of Claim 15, wherein said compound of the formula n-octyl C_6C_{12} glycopyranoside is selected from n-octyl β -D-glucopyranoside and n-octyl β -D-galactopyranoside.

17. The method of Claim 1, wherein said separating apparatus comprises a liquid phase separating apparatus.

18. The method of Claim 17, wherein said liquid phase separating apparatus comprises a reverse phase HPLC separating apparatus.

19. The method of Claim 18, wherein said reverse phase HPLC comprises non-porous reverse phase HPLC.

20. The method of Claim 1, wherein prior to said analyzing said first and second separated protein samples by mass spectroscopy, said first and second samples

are divided into first and second portions and wherein said second portions are subjected to enzymatic digestion.

21. The method of Claim 1, wherein said analyzing said first and second separated protein samples by mass spectrometry comprises analyzing said samples by
5 ESI or TOF/MS.

22. The method of Claim 1, wherein said analyzing said first and second separated protein samples by mass spectrometry comprises analyzing said samples by a technique selected from the group consisting of ion trap mass spectrometry, ion trap/time-of-flight mass spectrometry, quadrupole and triple quadrupole mass
10 spectrometry, Fourier Transform (ICR) mass spectrometry, and magnetic sector mass spectrometry.

23. A method, comprising:

a) providing:

i) a cell lysate derived from a cell of unknown type, said cell lysate comprising a plurality of proteins;
ii) a first protein profile map generated by the method of
15 Claim 1;
iii) a separating apparatus, wherein said separating apparatus separates proteins based on a physical property; and
iv) a mass spectroscopy apparatus; and

b) treating said cell lysate with said separating apparatus to produce a separated protein sample; wherein said separated protein sample is collected from said separating apparatus in a plurality of fractions, each of said fractions defined by a physical property;

c) analyzing said plurality of fractions from said separated protein sample with said mass spectroscopy apparatus to produce a second protein profile map; and

d) comparing said first protein profile map and said second protein profile map.

5 *Sub B1* 24. The method of Claim 23, wherein said first protein profile map displays protein abundance and mass from cell lysates of several known cell types and said second protein profile map displays protein abundance and mass from said cell lysate of unknown type.

10 *Sub B1* 25. The method of ~~Claim 24~~, wherein said protein abundance is expressed as bands of varying intensity.

15 *Sub B1* 26. The method of Claim 24, wherein said protein abundance is expressed as bands of different colors.

Sub B1 27. The method of Claim 24, wherein said protein abundance and mass are indicative of a cell identity.

28. A system comprising:

- a) a non-porous reverse phase HPLC separating apparatus;
- b) an automated sample handling apparatus configured to receive separated proteins from said reverse phase HPLC separating apparatus;
- c) a mass spectroscopy apparatus configured to receive proteins from said automated sample handling apparatus;
- d) a processor configured to produce a data representation of a protein profile map of separated proteins analyzed by said mass spectroscopy apparatus, wherein said protein profile map displays protein abundance and mass of a separated protein sample; and
- e) a display apparatus that displays said protein profile map.

29. The system of Claim 28, wherein said protein profile map displays protein abundance as bands of varying intensity.

30. The system of Claim 29, wherein said protein abundance is expressed as bands of different colors.

5 31. The system of Claim 28, wherein said protein abundance and mass are indicative of a cell type of said protein sample.

32. The system of Claim 28, wherein said processor is configured to determine the identity of individual bands on said protein profile map.

10 33. The system of Claim 28, wherein said automated sample handling device comprises a switchable, multi-channel valve.

34. The system of Claim 28, wherein said mass spectrometry apparatus comprises a ESI or TOF/MS apparatus.